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Antibiotic Resistance Patterns of Organisms Isolated from Cervico-Vaginal Mucus of Cows

V. S. PANANGALA AND
D. A. BARNUM*

INTRODUCTION

Antibacterial agents are widely used in chemotherapy of infections of the bovine female reproductive tract. The evaluation of results of treatment with chemotherapeutic agents in genital tract infections has been and will continue to be singularly difficult. The association of episome mediated transfer of drug resistance among certain bacterial strains (9, 16) and emergence of resistant mutants (8) presents a constantly changing pattern in susceptibility of organisms to the broad spectrum of antibiotics that are presently in use. Under most clinical situations, emperic treatment is instituted without recourse to microbiological laboratory investigation.

The most fundamental principle in the treatment of any infection is that therapy should be determined by isolation and determination of the causative organisms and *in vitro* testing of their susceptibility to antimicrobial agents (18). It is primarily important to know whether the pathogens isolated will be eliminated by concentrations of the antibiotic that can be safely achieved at the site of infection. Secondly, in order to avoid a protracted course of treatment, it should be determined whether any already instituted therapy may be altered if necessary.

This study presents the *in vitro* antibiotic resistance patterns of organisms isolated from the cervico-vaginal mucus of normal fertile and repeat breeder cows.

MATERIALS AND METHODS

The type of cows sampled and the methods used for isolation and characterization of the organisms from the cervico-vaginal mucus of the cows have been described earlier (10).

Bacterial isolates from each of the major groups *Micrococcaceae*, *Streptococcaceae*, *Enterobacteriaceae* and *Bacillaceae* were selected to represent at least one species from each herd examined. Isolates which represented genera that occurred in low frequencies were subjected to the antibiotic sensitivity test without any selection.

The standard Bauer-Kirby (3) single paper disc method was used in all the antibiotic sensitivity determinations. The medium used for the test was Mueller Hinton agar, poured to a depth of 4 mm in 150 × 15 mm plastic Petri plates. To support the growth of streptococci, *Corynebacterium pyogenes*, *Kurthia* spp. and *Neisseria* spp. media supplemented with 5% sterile calf serum were used. Representative plates from each batch were checked for sterility by incubating overnight at 37°C.

Sterile plates were seeded with standardized inoculum as suggested by Bauer and co-workers. Discs containing specific concentrations of the antimicrobial agents were applied onto the inoculated agar surface with a Sensi-Disc[1] dispenser. The anti-bacterial agents used with the respective disc potencies are listed in Table I.

The plates were incubated at 37°C. After overnight incubation (approximately 36 hours for *Corynebacterium pyogenes*), the zones of bacterial inhibition surrounding the discs were measured with calipers up to the nearest millimeter. The measurements were compared with the interpretive chart (17) and the zones graded as susceptible, intermediate, or resistant on the basis of the criteria presented.

*Department of Veterinary Microbiology and Immunology, University of Guelph, Guelph, Ontario, Canada N1G 2W1. Present address of senior author: Department of Immunology, New York State College of Veterinary Medicine, Ithaca, New York 14853. Reprint requests to junior author.

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[1]BBL, Division of Becton, Dickinson and Co., Canada, Ltd., Mississauga, Ontario.

TABLE I

ANTIMICROBIAL DRUGS[a] USED IN SUSCEPTIBILITY TESTING

Agent	Concentration
Lincomycin (L)	2 mcg
Methicillin (Me)	5 mcg
Ampicillin (AM)	10 mcg
Chloramphenicol (C)	30 mcg
Erythromycin (E)	15 mcg
Gentamycin (GM)	10 mcg
Novobiocin (NB)	30 mcg
Penicillin (P)	10 μ
Streptomycin (S)	10 mcg
Tetracycline (Te)	30 mcg
Bacitracin (B)	10 μ
Kanamycin (K)	30 mcg
Triple sulpha (SSS)[b]	1.0 mg
Colymycin (CL)	10 mcg

[a]BBL, Division of Becton, Dickinson & Co., Canada, Ltd. Mississauga, Ontario.

[b]Sulphadiazine, sulphamethazine and sulphamerazine.

RESULTS

The pattern of resistance to the 14 antibiotic agents used did not differ remarkably between isolates from the normal fertile and repeat breeder group to warrant a comparison between the groups. Therefore, the results of the susceptibility test for each species or genus were considered as a single entity irrespective of the group of animals to which they belonged.

Micrococcus species

The resistance pattern to 14 antibiotics for 24 isolates of *Micrococcus* species are presented in Table II. Of the 24 isolates listed, 54.2% were susceptible to lincomycin. Approximately 8% of the isolates were resistant to ampicillin while 34% were susceptible. Majority of the isolates (79.2%) were susceptible to erythromycin, the rest (20.8%) were categorized as intermediate. Resistance to novobiocin was shown by 30% of the isolates, 70% demonstrated an intermediate response and none were susceptible. Five percent of the isolates were resistant to penicillin, 79% showed an intermediate response and 16% were susceptible. The percentage susceptible to tetracycline was 58 and the rest, 42%, were resistant.

Staphylococcus species

Susceptibility to lincomycin and novobiocin were shown by 83.3% of the isolates of *S. epidermidis* and 16.7% gave zones of the intermediate category. Resistance to penicillin was shown by 67% of the isolates while 33% were of the intermediate type. The response to streptomycin was 75% susceptible, 21% resistant, and 4% intermediate. Fifty-eight percent of the isolates were susceptible to tetracycline and 10% demonstrated intermediate susceptibility. Resistance to triple sulpha was demonstrated by 13% of the isolates. Seventy-nine percent were susceptible and approximately 8% of the isolates gave an intermediate response. Susceptibility to methicillin, chloramphenicol, erythromycin, gentamycin, bacitracin and kanamycin were demonstrated by all isolates.

The two *S. aureus* isolates were resistant to tetracycline and colymycin, respectively, moderately susceptible to ampicillin, penicillin and streptomycin and were susceptible to all other antibiotics used.

Streptococcus species

The resistance profile of 43 isolates of *Streptococci* are shown in Table II. The isolates were comprised of *S. bovis* 12, *S. faecalis*, *S. faecium* 19 and *S. acidominimus* 12. Since there was no appreciable difference in response between the *S. faecalis* and *S. faecium* isolates to the 14 antibiotics, these two species were considered as representing a single group.

Susceptibility to lincomycin was shown by 92% of the *S. bovis* isolates. Resistance to gentamycin was demonstrated by 83.3% and 16.7% showed intermediate susceptibility. Ninety-two percent of the isolates were susceptible to novobiocin. Sixty-seven percent of the isolates were susceptible to tetracycline while 33% were resistant. The response to triple sulpha was 75% resistant, 17% intermediate, and 8% susceptible, respectively. In contrast to *S. bovis* isolates, certain differences in response were demonstrated by the 19 isolates representing *S. faecalis* and *S. faecium*. Susceptibility to chloramphenicol was demonstrated by all isolates and intermediate susceptibility to ampicillin. Approximately 95% of the isolates were resistant to lincomycin and 47% to tetracycline. The percentage susceptible to erythromycin and bacitracin were 95 and 63, respectively.

The *S. acidominimus* isolates were susceptible to a majority of the antibiotics used. Resistance to triple sulpha and colymycin was demonstrated by all isolates. Ninety-two percent of the isolates were resistant to kanamycin and 16.7% to streptomycin, respectively. Approximately 92% of the isolates were susceptible to gentamycin and 83% showed intermediate susceptibility to streptomycin.

Escherichia, *Proteus* and *Klebsiella* species

The 40 isolates of *E. coli* demonstrated a high degree of resistance to 50% of the antibiotic agents used. Resistance was demonstrated to lincomycin, methicillin, novobiocin, erythromycin, penicillin, bacitracin and colymycin by the total number of isolates. Seventy-eight percent were resistant to tetracycline. All isolates were susceptible to chloramphenicol, gentamy-

TABLE II

PREVALENCE OF DRUG RESISTANCE AMONG BACTERIA ISOLATED FROM CERVICO-VAGINAL MUCUS

Organism	Total Isolates Tested	Antimicrobial Drug													
		P	Me	AM	S	K	GM	NB	C	Te	L	E	B	CL	SSS
Micrococcus spp.	24	5[a]	0	8	0	0	0	30	0	42	0	0	0	100	0
S. epidermidis	24	67	0	54	21	0	0	0	0	41	0	0	0	100	13.0
S. aureus	2	0	0	0	0	0	0	0	0	100	0	0	0	100	0
Str. bovis	12	0	0	0	91	100	83	0	0	33	8	0	0	100	75.0
Str. faecalis and *Str. faecium*	19	100	100	0	100	100	100	15	0	47	94	0	0	100	100
Str. acidominimus	12	0	0	0	16	92	0	0	0	0	0	0	0	100	100
E. coliforms	3	100	100	9	3	0	0	100	0	80	100	100	100	100	2.5
Proteus spp.	5	60	100	0	60	0	0	20	0	100	100	100	100	100	60
B. licheniformis	17	0	0	0	0	0	0	5	0	5	100	0	94	100	0
B. firmus	12	0	8	0	41	0	0	33	0	8	83	0	100	100	0
B. pumilus	18	0	0	0	0	0	0	0	0	0	77	0	100	100	0
B. subtilus	2	0	0	0	0	0	0	0	0	0	100	0	100	100	0
C. pyogenes	23	0	0	0	96	8	8	0	4	8	4	17	0	100	100
Kurthia spp.	13	0	0	0	0	0	0	0	0	0	0	0	0	100	77
Neisseria spp.	2	0	100	0	50	0	0	0	0	0	100	0	0	0	0
Branhamella spp.	5	0	100	0	40	0	0	0	0	0	100	0	0	0	0
Acinetobacter spp.	3	0	33	0	100	0	0	0	0	0	100	100	0	0	33

[a]Percentage of strains of total tested resistant.
All strains were sensitive to chloramphenicol.

cin and kanamycin and approximately 90% demonstrated susceptibility to ampicillin and triple sulpha. Fifty-five percent of the isolates were susceptible to streptomycin, while 4.2% gave intermediate zones and 3% were completely resistant.

The response of the isolates representing the genus *Proteus* did not differ considerably from those produced by the *E. coli*. The five isolates belonging to this genus were resistant to lincomycin and colymycin to a comparable degree with *E. coli*. However, only 60% of the *Proteus* isolates were resistant to penicillin, streptomycin and triple sulpha, respectively.

The three isolates representing *Klebsiella* were resistant to lincomycin, methicillin, erythromycin, novobiocin, penicillin, tetracycline, bacitracin and colymycin, respectively.

Bacillus species

The *Bacillus* isolates were comprised of *B. licheniformis* 17, *B. firmus* 12, *B. pumilus* 18 and *B. subtilus* 2, respectively.

The 17 isolates of *B. licheniformis* were resistant to lincomycin and colymycin. The prevalence of resistance among the *B. firmus* isolates were comparable to the *B. licheniformis*. Of the 12 isolates, all were resistant to bacitracin and colymycin and 83% were also resistant to lincomycin.

In common with all the *Bacillus* species that were tested, *B. subtilus* and *B. pumilus* isolates were resistant to lincomycin, bacitracin and colymycin, respectively.

Corynebacterium pyogenes and *Kurthia* species

The prevalence of resistance among 23 isolates of *C. pyogenes* and 13 isolates of the genus *Kurthia* are presented in Table II. A consistent response was demonstrated by the 23 *C. pyogenes* isolates to six of the antibiotics employed. Considerable variations in the response to erythromycin, gentamycin, novobiocin, tetracycline and kanamycin were observed. Ninety-six percent of the isolates were resistant to streptomycin and the entire number was resistant to triple sulpha and colymycin.

The isolates representing the genus *Kurthia* were resistant to colymycin and 77% also demonstrated resistance to triple sulpha. The total number of isolates were susceptible to the remaining 12 antibiotics used.

Neisseria, *Branhamella*, *Acinetobacter* and *Pasteurella* species

The isolates representing the four genera occurred in very low numbers. The two isolates representing the genus *Neisseria* were resistant to lincomycin and methicillin. Both isolates gave an intermediate response to novobiocin and were susceptible to the remaining antibiotics.

The genus *Branhamella* represented by five isolates exhibited a difference in response to erythromycin and streptomycin, respectively. Two of the isolates were resistant to streptomycin, while two demonstrated an intermediate response and one was susceptible.

The genus *Acinetobacter* was represented by three isolates. Resistance to lincomycin, erythromycin and streptomycin were demonstrated by all three. One of the isolates was resistant to triple sulpha, while two demonstrated zones of the intermediate category.

The genus *Pasteurella* was represented by a single isolate. Resistance was demonstrated to lincomycin and colymycin. Inhibition zones of the intermediate range were produced against streptomycin, bacitracin and triple sulpha.

A graphic representation of the resistance profile of the 237 bacterial isolates to the 14 antibiotics employed is shown in Figure 1.

DISCUSSION

In the total sample, about two thirds of the isolates are resistant to one or more of the antibiotic agents used. The range of resistance varies from 0.4% for chloramphenicol to 95.8% for colymycin. An assessment of the extent of resistance to the 14 antibiotics used reveals a gradient of the following order: colymycin 95.8%, lincomycin 51.5%, bacitracin 40.5%, penicillin 34.6%, triple sulpha 34.2%, methicillin 32.1%, streptomycin, 31.2%, tetracycline 28.7%, novobiocin 24.9%, erythromycin 23.2%, kanamycin 18.6%, gentamycin 13.1%, ampicillin 8.0% and chloramphenicol 0.4%, respectively. The broadest spectrum of inhibitory activity is demonstrated by chloramphenicol, closely followed by gentamycin, kanamycin, ampicillin and erythromycin.

The use of antibiotics in the treatment of bovine genital infections has been reported by several workers (12, 13, 14), but the results are contradictory. In many cases, potentially useful bioactive compounds have lost their significance for therapy due to extensive and prolonged use. Outstanding examples of such an occurrence is the emergence of penicillin-resistant strains of gonococci (5) and pyogenic staphylococci (2) in man.

A high degree of resistance among *E. coli* isolated in the present study is demonstrated against all except chloramphenicol, gentamycin, streptomycin, kanamycin and triple sulpha. Comparable resistance patterns are also observed in *Proteus* and *Klebsiella* species isolated. Transferable drug resistance among most genera of the family *Enterobacteriaceae* is well documented (6, 16). Much impetus has been given to the subject by the concern of the spread of infectious resistance among intestinal bacteria in farm animals and thence to man (1, 15). The frequent existence of multiple infectious resistance in *E. coli* from calves and pigs has been reported by

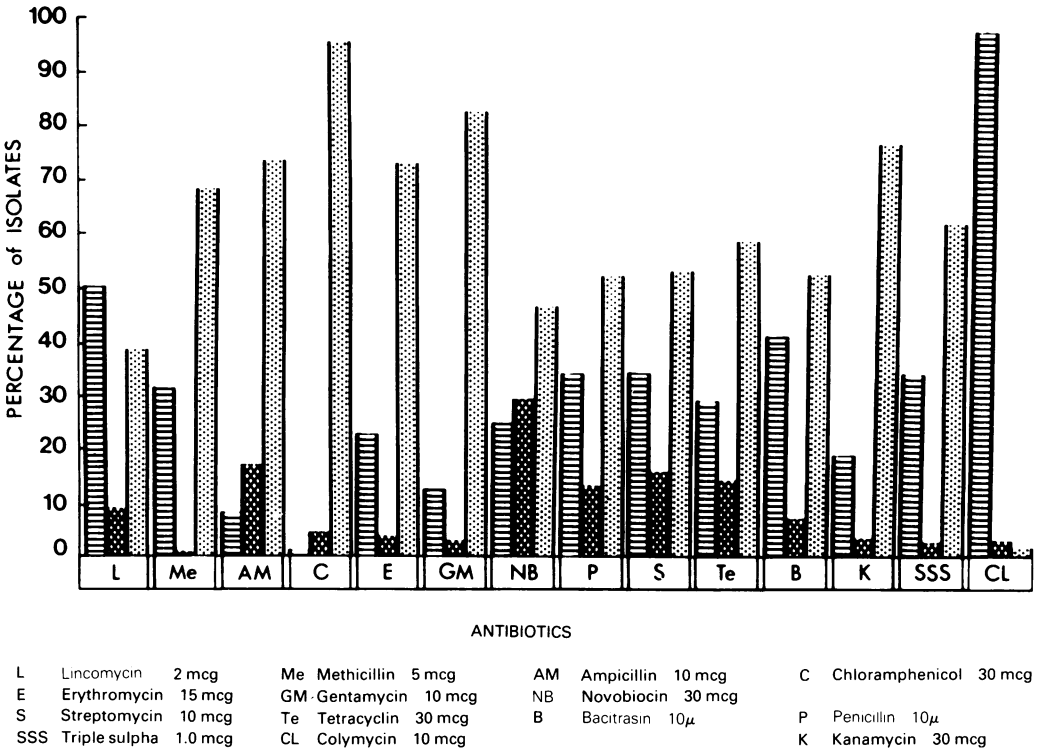

FIGURE 1. Antibiotic susceptibility *in vitro* of 237 bacterial isolates from cervico-vaginal mucus of cows. Legend: Crossbars – resistant strains, hatched – intermediate resistance and stippled – susceptible.

Walton (15). Excessive use of antibiotics in livestock, particularly large scale administration in the form of feed supplements, are partly responsible for the free dissemination of multiple drug resistance among bacteria. The potential hazards to human health by the excretion of drug residues in milk (7, 11) must also be taken into consideration when antibiotics are used in the treatment of uterine infections.

Although the effectiveness of antibiotics depends on many factors, only two of them lend themselves to objective quantification. These are the sensitivity of the causative agents of infection to antibiotics and the latter's concentration in the inflammatory focus. For many microorganisms, the degree of susceptibility to antibiotics can be determined *in vitro*. In some cases, the resistance to bacterial species is natural not acquired. The interpretive schemes for monitoring microbial susceptibility to antimicrobial agents should be based on recognized standards so that results obtained could be correlated with the response to therapy observed in clinical practice.

"Most published data on antimicrobial sensitivities of pathogenic microorganisms of animal origin antedate the introduction and adoption of standardized testing methods in the course of routine laboratory diagnosis" (4). The Bauer-Kirby technique used in the present study is currently considered satisfactorily standardized and is used in determining antibiotic susceptibility in veterinary diagnostic laboratories in Ontario.

SUMMARY

The resistance patterns of 237 bacterial isolates from the cervico-vaginal mucus of cows to 14 antibiotics were determined. The organisms included genera belonging to four major families, *Micrococcaceae*, *Streptococcaceae*, *Enterobacteriaceae*, *Bacillaceae* and a few genera, namely, *Corynebacterium*, *Kurthia*, *Neisseria*, *Branhamella*, *Acinetobacter* and *Pasteurella* which occurred in low frequency.

An assessment of the extent of resistance to the 14 antibiotics used revealed a gradient of the following order: colymycin 95.8%, lincomycin 51.5%, bacitracin 40.5%, penicillin 34.6%, triple sulpha 34.2%, methicillin 32.1%, streptomycin 31.2%, tetracycline 28.7%, novobiocin 24.9%, erythromycin 23.2%, kanamycin 18.6%, gentamycin 13.1%, ampicillin 8.0% and chloramphenicol 0.4%.

The use of standardized techniques for monitoring microbial susceptibility to antimicrobial agents is emphasized.

RÉSUMÉ

Les auteurs ont déterminé le profil de la résistance de 237 souches de bactéries isolées du

mucus cervico-vaginal de vaches, à l'endroit de 14 antibiotiques. Ces bactéries incluaient des genres appartenant aux quatre familles importantes suivantes: *Micrococcaceae, Streptococcaceae, Enterobacteriaceae* et *Bacillaceae*; elles comprenaient aussi des genres dont l'incidence se révéla moins fréquente, entre autres: *Corynebacterium, Kurthia, Neisseria, Branhamella, Acinetobacter* et *Pasteurella*.

La détermination de l'étendue de la résistance aux 14 antibiotiques expérimentaux donna des résultats dont la décroissance se manifesta ainsi: colymycine 95.8%, lincomycine 51.5%, bacitracine 40.5%, pénicilline 34.6%, triple sulfa 34.2%, méthicilline 32.1%, streptomycine 31.2%, tétracycline 28.7%, novobiocine 24.9%, érythromycine 23.2%, kanamycine 18.6%, gentamycine 13.1%, ampicilline 8% et le chloramphénicol 0.4%.

Les auteurs insistent sur l'utilisation de techniques standardisées pour vérifier la sensibilité des bactéries à l'endroit des antibiotiques.

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